

PROTEIN RECOVERY

RELATED APPLICATION

This application is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application number 60/050,951 filed Jun. 13, 1997, the contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to protein recovery. In particular, it pertains to recovery of a polypeptide, wherein the polypeptide is exposed to an immobilized reagent which binds to, or modifies, the polypeptide.

2. Description of Related Art

The large-scale, economic purification of proteins is increasingly an important problem for the biotechnology industry. Generally, proteins are produced by cell culture, using either mammalian or bacterial cell lines engineered to produce the protein of interest by insertion of a recombinant plasmid containing the gene for that protein. Since the cell lines used are living organisms, they must be fed with a complex growth medium, containing sugars, amino acids, and growth factors, usually supplied from preparations of animal serum. Separation of the desired protein from the mixture of compounds fed to the cells and from the by-products of the cells themselves to a purity sufficient for use as a human therapeutic poses a formidable challenge.

Procedures for purification of proteins from cell debris initially depend on the site of expression of the protein. Some proteins can be caused to be secreted directly from the cell into the surrounding growth media; others are made intracellularly. For the latter proteins, the first step of a purification process involves lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are generally removed by differential centrifugation or by filtration. The same problem arises, although on a smaller scale, with directly secreted proteins due to the natural death of cells and release of intracellular host cell proteins in the course of the protein production run.

Once a clarified solution containing the protein of interest has been obtained, its separation from the other proteins produced by the cell is usually attempted using a combination of different chromatography techniques. These techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, or size. Several different chromatography resins are available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, being then differentially eluted by different solvents. In some cases, the desired protein is separated from impurities when the impurities specifically adhere to the column, and the protein of interest does not, that is, the protein of interest is present in the "flow-through."

As part of the overall recovery process for the protein, the protein may be exposed to an immobilized reagent which

binds to or modifies the protein. For example, the protein may be subjected to affinity chromatography wherein an immobilized reagent which binds specifically to the protein, such as an antibody, captures the antibody and impurities pass through the affinity chromatography column. The protein can be subsequently eluted from the column by changing the conditions such that the protein no longer binds to the immobilized reagent. The immobilized reagent may also be an enzyme which modifies the protein. Sahni et al., *Anal. Biochem.* 193:178-185 (1991) and Voyksner et al., *Anal. Biochem.* 188:72-81 (1990) describe immobilized proteases.

Another type of purification process is filtration. Filtration of fine particle size contaminants from fluids has been accomplished by the use of various porous filter media through which a contaminated composition is passed such that the filter retains the contaminant. Retention of the contaminant may occur by mechanical straining or electrokinetic particle capture and adsorption. In mechanical straining, a particle is retained by physical entrapment when it attempts to pass through a pore smaller than itself. In the case of electrokinetic capture mechanisms, the particle collides with a surface within the porous filter and is retained on the surface by short range attractive forces. To achieve electrokinetic capture, charge modifying systems can be used to alter the surface charge characteristics of a filter (see, e.g., W090/11814). For example, where the contaminant to be removed is anionic, a cationic charge modifier can be used to alter the charge characteristics of the filter such that the contaminant is retained by the filter.

There is a need in the art for improved methods for recovering polypeptides, especially those polypeptides produced by recombinant techniques.

SUMMARY OF THE INVENTION

Accordingly, the invention provides a method for recovering a polypeptide comprising: (a) exposing a composition comprising a polypeptide to a reagent which binds to, or modifies, the polypeptide, wherein the reagent is immobilized on a solid phase; and then (b) passing the composition through a filter bearing a charge which is opposite to the charge of the reagent in the composition, so as to remove leached reagent from the composition. Preferably the charge characteristics of the polypeptide in the composition in step (b) are such that the polypeptide passes through the filter and preferably the filter is placed in line with the composition exposed to the reagent as in step (a). In one embodiment of the invention, the polypeptide to be treated in step (a) is a precursor polypeptide and the immobilized reagent is a protease (e.g. pepsin) which removes a precursor domain (e.g. a leucine zipper dimerization domain) from the polypeptide.

The invention also provides a method for recovering a polypeptide comprising removing a leached reagent from a composition comprising the polypeptide and the leached reagent by passing the composition through a filter bearing a charge opposite to that of the leached reagent, wherein the leached reagent was previously immobilized on a solid phase.

In yet a further embodiment, the invention provides a method for modifying a precursor antibody comprising a leucine zipper dimerization domain, comprising exposing the precursor antibody to a protease immobilized on a solid phase such that the protease removes the leucine zipper from the precursor antibody. This method optionally further comprises passing the antibody free of the leucine zipper through